> would most probably scatter light in dark field microscopy, whereas under such examination the ground substance is normally quite clear and nonrefractile. (pp. 127–8)

Early electron microscopists drew upon the armory of fixatives already employed in light microscopy. In his early studies of electron microscopy with separated fractions, Claude explored the potential of a variety of fixatives (formaldehyde, potassium dichromate, osmium tetroxide). Likewise, he and Porter, in their first study with tissue-cultured cells also tried several fixatives (chromic acid, acid formaldehyde, Flemming's mixture) and concluded that osmium tetroxide (OsO_4) vapors or solutions generated the clearest and most detailed images. Formaldehyde, they found, failed to show cytoplasmic structures, while chromic acid caused the cytoplasm to shrink and revealed only very small granules in it, and Flemming's solution yielded granules and mitochondria with a very coarse appearance. They claimed that alcohol, acetic acid, and freeze-drying (see below) were even worse in producing images resembling living cells. The standard, thus, was whether the resulting micrographs looked the way researchers expected them to look.

From the outset of electron microscopy, osmium tetroxide was by far the most widely used fixative. It had a long history of use in light microscopy, having been first introduced by Max Shultze in 1865 in his studies of the marine protozoan, *Noctiluca*. Strangeways and Canti (1927) had compared it with several other classical fixatives and concluded that it produced the least detectable change, the most evident being an increase in light scattering by the nucleus, which they attributed to the development of a "fine precipitate" (p. 9).

One way of responding to the worries about chemical fixation relied on the use of an alternative preparation technique, freeze-drying, which we have already briefly encountered in the work of Altmann and also of Bensley. The technique had been pioneered by Altmann (1890) who froze small pieces of tissue and kept them over sulfuric acid in vacuo at a temperature of –20° C for some days. The water dissipates directly into a gaseous state so that there is no intervening liquid phase that might distort the cell, as there is in chemical fixation. The result is a progressive dehydration of the tissue. Bensley and Gersh revived and improved Altmann's technique, in large part by freezing to even colder temperatures.[33] Bensley and Gersh (1933a, p. 212) argued that

[33] Gersh (1932) described the principles underlying the technique: "In the freeze-drying method . . . the system is cooled very rapidly to such a low temperature [−40°C] that the cohesive effects existing between ambient molecules at the instant of cooling predominate over all others; ideally all molecules are literally frozen in their tracts; they have no mobility; only occasionally does the translational energy of a surface molecule become great enough for it to escape; then it